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Helicobacter pylori live vaccine

Specification

5

The present invention relates to novel recombinant live vaccines, which provide protective immunity against an infection by *Helicobacter pylori* and a method of screening *H. pylori* antigens for optimized vaccines.

10

Helicobacter is a gram-negative bacterial pathogen associated with the development of gastritis, peptic ulceration and gastric carcinoma. Several *Helicobacter* species colonize the stomach, most notably *H. pylori*, *H. heilmanii* and *H. felis*.

15 Although *H. pylori* is the species most commonly associated with human infection, *H. heilmanii* and *H. felis* also have been found to infect humans. High *H. pylori* infection rates are observed in third world countries, as well as in industrialized countries. Among all the virulence factors described in *H.*
20 *pylori*, urease is known to be essential for colonisation of gnotobiotic pigs and nude mice. Urease is an enzyme composed of two structural subunits (UreA and UreB). Previous studies have indicated that oral immunization using recombinant UreB plus cholera toxin were able to protect mice from gastric colonisa-
25 tion with *H. felis* and *H. pylori* (Michetti et al., Gastroenterology 107 (1994), 1002-1011). By oral administration of recombinant UreB antigens, however, in several cases only an incomplete protection can be obtained. Other *H. pylori* antigens shown to give partial protection are the 87 kD vacuolar
30 cytotoxin VacA (Cover and Blaser, J. Biol. Chem. 267 (1992), 10570; Marchetti et al., Science 267 (1995), 1655) and the 13 and 58 kD heat shock proteins HspA and HspB (Ferrero et al., Proc. Natl. Acad. Sci. USA 92 (1995), 6499).

35 Attenuated pathogens, e.g. bacteria, such as *Salmonella*, are known to be efficient live vaccines. The first indications of the efficacy of attenuated *Salmonella* as good vaccine in hu-

mans came from studies using a chemically mutagenized *Salmonella typhi* Ty21a strain (Germanier and Furer, J. Infect. Dis. 141 (1975), 553-558), tested successfully in adult volunteers (Gilman et al., J. Infect. Dis. 136 (1977), 717-723) and later
5 on in children in a large field trial in Egypt (Whadan et al., J. Infect. Dis. 145 (1982), 292-295). The orally administered Ty21a vaccine was able to protect 96% of the Egyptian children vaccinated during three years of surveillance. Since that time new attenuated *Salmonella* live vector vaccines have developed
10 (Hone et al., Vaccine 9 (1991), 810-816), in which well defined mutations incorporated into the chromosome gave rise to non-virulent strains able to induce strong immune responses after oral administration (Tacket et al., Vaccine 10 (1992), 443-446 and Tacket et al., Infect. Immun. 60 (1992), 536-541).
15 Other advantages of the live attenuated *Salmonella* vaccine include its safety, easy administration, long-time protection and no adverse reactions in comparison with the former inactivated wholesale typhoid vaccines (Levine et al., Typhoid Fever Vaccines. In: Plotkin S.A., Mortimer E.A. Jr. (eds.) Vaccines. Philadelphia: WB Saunders (1988), 333-361).
20

Mutants of *S. typhimurium* have been extensively used to deliver antigens because of the possibility to use mice as an animal model, which is believed to mimic *S. typhi* infections
25 in humans. The attenuation of *S. typhimurium* most commonly used consists in site directed mutagenesis of genes affecting the synthesis of aromatic amino acids. Such strains, designated aro mutants, have a negligible pathogenicity, as demonstrated in animal models and human trials using these constructs
30 (Hoiseth and Stocker, Nature 291 (1981), 238-239; Tacket et al. (1992), Supra). Advantage has been taken from the potent immunogenicity of live *Salmonella* vaccine to deliver heterologous antigens. Expression of specific antigens in attenuated *Salmonella* has conferred murine protection against several
35 bacterial pathogens. The use of recombinant live vaccines, which are capable of expressing *Helicobacter* antigens and protecting the vaccinated animals, has not yet been described.

The use of attenuated live vaccines for the treatment of a Helicobacter infection has also not been rendered obvious. The reason therefor being that in the course of the Helicobacter infection a strong immune response against the pathogen per se is induced, which, however, does not lead to a protective immunity. Thus, it was highly surprising that a protective immune response is achieved when using recombinant attenuated bacterial cells as antigen carriers, which are capable of expressing a DNA molecule encoding a Helicobacter antigen. Apparently, recombinant attenuated bacterial cells expressing a Helicobacter antigen are capable of creating a qualitatively different immune response against the heterologous Helicobacter antigen than Helicobacter itself does against its own homologous antigen. Surprisingly, a non-protective immune response is thus transformed into an immune response protecting against Helicobacter infections. This unexpected observation renders it possible to use recombinant attenuated pathogens, e.g. bacterial cells, particularly Salmonella, as carriers for the screening of protective antigens, to apply the protective antigens identified in this manner in any vaccine against Helicobacter infections, and to use recombinant attenuated bacteria as carriers of protective antigens for the immunization against Helicobacter infections in humans and other mammals.

Thus, a subject matter of the present invention is a recombinant attenuated pathogen, which comprises at least one heterologous nucleic acid molecule encoding a Helicobacter antigen, wherein said pathogen is capable to express said nucleic acid molecule or capable to cause the expression of said nucleic acid in a target cell. Preferably the nucleic acid molecule is a DNA molecule.

The attenuated pathogen is a microorganism strain which is able to cause infection and preferably effective immunological protection against the actual pathogen but is no longer pathogenic per se. The attenuated pathogen can be a bacterium, a

virus, a fungus or a parasite. Preferably it is a bacterium; e.g. *Salmonella*, such as *S. typhimurium* or *S. typhi*, *Vibrio cholerae* (Mekalanos et al., *Nature* 306 (1983), 551-557), *Shigella* Species such as *S. flexneri* (Sizemore et al., *Science* 5 270 (1995), 299-302; Mounier et al., *EMBO J.* 11 (1992), 1991-1999), *Listeria* such as *L. monocytogenes* (Milon and Cossart, *Trends in Microbiology* 3 (1995), 451-453), *Escherichia coli*, *Streptococcus*, such as *S. gordonii* (Medaglini et al., *Proc. Natl. Acad. Sci. USA* 92 (1995) 6868-6872) or *Mycobacterium*,
10 such as *Bacille Calmette Guerin* (Flynn, *Cell. Mol. Biol.* 40 Suppl. 1 (1994), 31-36). More preferably the pathogen is an attenuated enterobacterium such as *Vibrio cholerae*, *Shigella flexneri*, *Escherichia coli* or *Salmonella*. Most preferably the attenuated pathogen is a *Salmonella* cell, e.g. a *Salmonella*
15 *aro* mutant cell. The attenuated pathogen, however, can be a virus, e.g. an attenuated *vaccinia* virus, *adenovirus* or *pox* virus.

The nucleic acid molecule which is inserted into the pathogen
20 codes for a *Helicobacter* antigen, preferably a *H. felis*, *H. heilmanii* or *H. pylori* antigen, more preferably a *H. pylori* antigen. The *Helicobacter* antigen can be a native *Helicobacter* polypeptide, an immunologically reactive fragment thereof, or an immunologically reactive variant of a native polypeptide or
25 of a fragment thereof. Further, the *Helicobacter* antigen can be a protective carbohydrate or a peptide mimotope simulating the three-dimensional structure of a native *Helicobacter* antigen. Peptide mimotopes can be obtained from peptide libraries presented on the surface of bacterial cells (cf. PCT/EP96/
30 01130). Of course, the transformed cell can also contain several DNA molecules coding for different *Helicobacter* antigens.

Attenuated bacteria can be used to transcribe and translate
35 said nucleic acid molecule directly in the bacterial cell or to deliver said nucleic acid molecule to the infected target cell, such that the DNA molecule is transcribed and/or trans-

lated by the eukaryotic target cell machinery. This indirect bacterial vaccination procedure, termed here as genetic vaccination, has been successfully used with Shigella as a carrier (Sizemore, D. R., Branstrom, A. A. & Sadoff, J. C. (1995) Attenuated Shigella as a DNA delivery vehicle for DNA-mediated immunization. Science 270:299-302).

In a preferred embodiment of the present invention the Helicobacter antigen is urease, a urease subunit or an immunologically reactive variant or fragment thereof or a peptide mimotope thereof. In a further preferred embodiment of the present invention the Helicobacter antigen is a secretory polypeptide from Helicobacter, an immunologically reactive variant or fragment thereof or a peptide mimotope thereof. A process for identifying Helicobacter genes coding for such secretory polypeptides, and particularly for adhesins, has been disclosed in the international patent application PCT/EP96/02544, which is incorporated herein by reference. This process comprises

- a) preparing a gene bank of H. pylori DNA in a host organism containing an inducible transposon coupled to a marker of secretory activity,
- b) inducing the insertion of the transposon into the H. pylori DNA and
- c) conducting a selection for clones containing a secretory gene by means of the marker, and optionally further
- d) conducting a retransformation of H. pylori by means of the DNA of clones containing genes having secretory activity, wherein isogenic H. pylori mutant strains are produced by means of integrating the DNA into the chromosome, and
- e) conducting a selection detecting adherence-deficient H. pylori mutant strains.

Suitable examples of antigens obtainable by the above process are selected from the group consisting of the antigens AlpA, AlpB, immunologically reactive variants or fragments thereof

or peptide mimotopes thereof. The nucleic and amino acid sequences of the antigens AlpA and AlpB have been disclosed in the international patent applications PCT/EP96/02545 and PCT/EP96/04124, which are incorporated herein by reference. Further, the nucleic and amino acid sequences of AlpB are shown in SEQ ID NO. 1 and 2, and the nucleic and amino acid sequences of AlpA in SEQ ID NO. 3 and 4.

It is also conceivable, however, that an intracellular antigen is used which can be presented on the surface, e.g. by autolytic release, and confers immunological protection.

The presentation of the Helicobacter antigens in the recombinant pathogen according to the invention can be accomplished in different ways. The antigen or the antigens can be synthesized in a constitutive, inducible or phase variable manner in the recombinant pathogen. Concerning the constitutive or inducible synthesis of the Helicobacter antigens known expression systems can be referred to, as have been described by Sambrook et al., Molecular Cloning, A Laboratory Manual (1989), Cold Spring Harbor Laboratory Press.

Particularly preferred the antigens are presented in a phase variable expression system. Such a phase variable expression system for the production and presentation of foreign antigens in hybrid live vaccines is disclosed in EP-B-0 565 548, which is herein incorporated by reference. In such a phase variable expression system the nucleic acid molecule encoding the Helicobacter antigen is under control of an expression signal, which is substantially inactive in the pathogen, and which is capable of being activated by a spontaneous reorganization caused by a nucleic acid, e.g. DNA reorganization mechanism in the pathogen, e.g. a specific DNA inversion process, a specific DNA deletion process, a specific DNA replication process or a specific slipped-strand-mispairing mechanism.

A recombinant cell having a phase variable expression system

is capable of forming two subpopulations A and B, wherein the division into said subpopulations occurs by spontaneous reorganization in the recombinant nucleic acid, wherein said subpopulation A is capable of infection and immunologically active per se, while subpopulation B, which is regenerated from subpopulation A, produces at least one heterologous Helicobacter antigen and acts immunologically with respect to said additional antigen.

10 The activation of the expression signal encoding the Helicobacter antigen can be directly accomplished by nucleic acid reorganization or, alternatively, indirectly accomplished by activation of a gene encoding a protein which controls the expression of the gene encoding the Helicobacter antigen. The
15 indirect activation represents a system which allows the production of the Helicobacter antigen via a cascade system, which can be realized e.g. in that the gene directly controlled by DNA reorganization codes for an RNA polymerase which is specific for the promoter preceding the Helicobacter gene, or
20 a gene regulator which in another specific manner induces the expression of the Helicobacter gene. In an especially preferred embodiment of the present invention the expression signal for the gene encoding the Helicobacter antigen is a bacteriophage promoter, e.g. a T3, T7 or SP6 promoter, and the activation
25 of the expression signal is caused by a nucleic acid reorganization resulting in the production of a corresponding bacteriophage RNA polymerase in the pathogen.

The phase variable expression system can be adjusted to provide a preselected expression level of the Helicobacter antigen. This can be accomplished e.g. by modifying the nucleotide sequence of the expression signal, which is activated by the nucleic acid reorganization mechanism, and/or by inserting further genetic regulation elements.

35 The Helicobacter antigens can be produced in an intracellular, as well as in an extracellular manner in the pathogen accord-

ing to the invention. For instance, autotransporter systems such as the IgA-protease system (cf. for instance EP-A-0 254 090) or the E. coli AIDA-1 adhesin system (Benz et al., Mol. Microbiol. 6 (1992), 1539) are suited as extracellular secretory system. Other suitable outer membrane transporter systems are the RTX-toxin transporters, e.g. the E. coli hemolysin transport system (Hess et al., Proc. Natl. Acad. Sci. USA 93 (1996), 11458-11463).

10 The pathogen according to the invention can contain a second heterologous nucleic acid, e.g. DNA molecule, which codes for an immunomodulatory polypeptide influencing the immune response quantitatively or qualitatively, apart from the nucleic acid molecule encoding the Helicobacter antigen. Examples of
15 such immunomodulatory polypeptides are immune-stimulating peptides, cytokines like IL-2, IL-6 or IL-12, chemokines, toxins, such as cholera toxin B or adhesins.

The present invention also refers to a pharmaceutical composition comprising as an active agent a recombinant attenuated
20 pathogen as described above, optionally together with pharmaceutically acceptable diluents, carriers and adjuvants. Preferably, the composition is a living vaccine. The vaccination routes depend upon the choice of the vaccination vector. The
25 administration may be achieved in a single dose or repeated at intervals. The appropriate dosage depends on various parameters such as the vaccinal vector itself, or the route of administration. Administration to a mucosal surface (e.g. ocular, intranasal, oral, gastric, intestinal, rectal, vaginal or
30 urinary tract) or via the parenteral route (e.g. subcutaneous, intradermal, intramuscular, intravenous or intraperitoneal) might be chosen. A method for the preparation of the living vaccine comprises formulating the attenuated pathogen in a pharmaceutically effective amount with pharmaceutically acceptable
35 diluents, carriers and/or adjuvants.

Further, the present invention refers to a method for prepar-

ing a recombinant attenuated pathogen as defined above, comprising the steps of a) inserting a nucleic acid molecule encoding a Helicobacter antigen into an attenuated pathogen, wherein the recombinant pathogen, e.g. a transformed bacterial
5 cell, is obtained, which is capable of expressing said nucleic acid molecule or is capable to cause expression of said nucleic acid molecule in a target cell and b) cultivating said recombinant attenuated pathogen under suitable conditions. If the pathogen is a bacterial cell, the nucleic acid molecule
10 encoding the Helicobacter antigen can be located on an extra-chromosomal plasmid. It is, however, also possible to insert the nucleic acid molecule into the chromosome of the pathogen.

Furthermore, the present invention refers to a method for
15 identifying Helicobacter antigens which raise a protective immune response in a mammalian host, comprising the steps of: a) providing an expression gene bank of Helicobacter in an attenuated pathogen and b) screening the clones of the gene bank for the ability to confer a protective immunity against a
20 Helicobacter infection in a mammalian host. Preferably, this identification process takes place in a phase variable expression system, rendering possible a stable expression of all of the Helicobacter antigens. Recombinant clones can then be applied as "pools" for the oral immunization of test animals,
25 such as mice. The potential of these clones as protective antigen is then determined via a challenge infection with Helicobacter, e.g. a mouse-adapted H. pylori strain. Thus, there is a possibility of directly selecting optimized H. pylori vaccine antigens.

30

The invention will be further illustrated by the following figures and sequence listings.

Fig. 1: shows a schematic illustration of the urease expression vector pYZ97; whereon the genes coding for the
35 urease subunits UreA and UreB are located under transcriptional control of the T7 promoter $\phi 10$.

There is a ribosomal binding site (RBS) between the T7 promoter and the urease genes. Further, the plasmid exhibits an origin of replication (ori), a β -lactamase resistance gene (bla) and 4 T7 terminators in series.

Apart from the expression by the T7 promoter, a constitutive low level expression of the urease A and B subunits can also be brought about via a cryptic promoter, which is located upstream from the T7 promoter, on the plasmid pYZ97.

Fig. 2: shows a schematic illustration of the T7 RNA polymerase (T7RNAP) expression cassettes pYZ88, pYZ84 and pYZ114, which can be integrated into the chromosomes of bacteria.

In the high-expression cassette pYZ88 the lambda PL promoter is located in inverse orientation, upstream from the T7RNAP gene. A gene for the temperature-sensitive repressor cI 857 (cI) is under control of this promoter. A terminator of the bacteriophage fd (fdT) is situated upstream from the cI gene. The gin gene (Mertens, EMBO J. 3 (1984), 2415-2421) codes for a control enzyme of a DNA reorganization mechanism. A DNA sequence coding for the tRNA Arg is located downstream from the gin gene.

In phase A the PL promoter responsible for the expression of the T7RNAP gene is directed in the direction of the cI857 gene and the gin gene. The consequence of this is that an active repressor is formed at the permissive temperature of 28°C and reduces the transcription from the PL promoter. At a higher temperature the transcription of the PL promoter is increased, since the repressor is inactivated at least partially under such external influences. The

temperature-dependent increase in the transcription also causes a corresponding increase in the expression of the following *gin* gene, which as a control enzyme catalyses the inversion of the PL promoter and the transition in phase B, in which the T7RNAP gene is expressed.

In the high-expression system pYZ88 a further fdT transcription terminator is located between a kanamycin-resistance gene (*km*) and the promoter of this gene. In this manner, the synthesis of an anti-sense RNA, inversely orientated to the T7RNAP gene, which normally contributes to the reduction of the T7RNAP expression, is reduced. This results in a high expression of the T7RNAP.

In the medium-expression system pYZ84 a transcription terminator (fdT) is located between the PL promoter and the start of the T7RNAP gene. In this manner the expression of the T7RNAP mRNA is reduced. Additionally, the anti-sense RNA affects the T7RNAP translation. Therefore, only a medium expression occurs.

In the low-expression system pYZ114 a deletion of 100 bp in PL is additionally introduced (Δ PL). In this manner the activity of the PL promoter is reduced to a high extent, which leads to a lower T7RNAP expression and thus to a reduction of the UreA/B gene expression. In this construct the effect of the cryptic promoter on pYZ97 is already observed.

SEQ ID NO. 1 and 2 show the nucleotide sequence of the adhesin gene *AlpB* from *H. pylori* and the amino acid sequence of the polypeptide coded therefrom.

SEQ ID NO. 3 and 4 show the nucleotide sequence of the adhesin

gene AlpA from *H. pylori* and the amino acid sequence of the protein coded therefrom.

Experimental part

5

Materials and Methods

Bacterial strains: *S. typhimurium* SL3261 live vector vaccine strain was used as a recipient for the recombinant *H. pylori* urease plasmid constructs. *S. typhimurium* SL3261 is an *aroA* transposon mutant derived from *S. typhimurium* SL1344 wild type strain. *S. typhimurium* SL3261 is a non-virulent strain that gives protection to mice against infection with wild type *S. typhimurium* after oral administration (Hoiseth and Stocker
10 (1981) Supra). *S. typhimurium* SL3261 and derivatives thereof, which contain the urease expression plasmid pYZ97 (extrachromosomal) and the T7RNAP expression cassettes pYZ88, pYZ84 or pYZ114, respectively (integrated into the chromosome) are indicated in table 1. Luria broth or agar was used for bacterial growth at 28°C. *H. pylori* wild type strain grown at 37°C
15 on serum plates was used for the challenge experiments.

Immunization of mice: Four weeks Balb/c mice purchased from Interfauna (Tuttlingen, Germany) were adapted two weeks in an
25 animal facility before being used for experimentation. 150 µl of blood was taken retroorbitally from all mice to obtain preimmune serum. Retroorbital bleedings were repeated from all immunized mice 1 week and 3 weeks after immunization.

30 Eight groups of 5 mice including controls were used in this study (table 2). Group A, the naive control group, was not immunized with *Salmonella* neither challenged with wild type *H. pylori*. The rest of the groups were all orally immunized. Group B, a negative control group, did not receive *Salmonella*
35 and was challenged with *H. pylori*. Mice from groups C to G were immunized with *Salmonella* vaccine strains and challenged with *H. pylori*. The last group H received recombinant urease B

in combination with cholera toxin and was also challenged.

Prior to immunizations mice were left overnight without solid food and 4 hours without water. 100 μ l of 3% sodium bicarbonate were given orally using a stainless steel catheter tube to neutralize the stomach pH. Then mice from group B received 100 μ l PBS and mice from groups C to G received 1.0×10^{10} CFU of *Salmonella* in a 100 μ l volume. Mice from group H received four times 100 μ l of a mixture of recombinant *H. pylori* Ure-aseB plus cholera toxin, one dose every week. After every immunization water and food were returned to the mice.

H. pylori challenge: Four weeks after the first oral immunization mice from groups B to H were challenged with *H. pylori*. Mice were left overnight without solid food and without water 4 hours prior to the challenge. 100 μ l of 3% sodium bicarbonate were given orally to the mice using a stainless steel catheter tube, followed by an oral dose of 5.0×10^9 CFU/ml of *Helicobacter pylori*. Water and food were returned to the mice after the challenge.

Collection of blood and tissues from mice: Twelve weeks after the first immunization the mice were left overnight without food and subsequently sacrificed for analysis of protection and immune response. The mice were anaesthetized with Metoxy-fluorane for terminal cardiac bleeding and prior to sacrifice by cervical dislocation. Under aseptic conditions, spleen and stomach were carefully removed from each mouse and placed on ice in separate sterile containers until further processing. Large and small intestine were obtained for further isolation of the intestinal fluid.

Processing of stomach and measurement of urease activity: The degree of *H. pylori* colonisation in the mouse stomach was measured by the presence of active urease in the tissue. The Jatrox-test (Röhm-Pharma GmbH, Weiterstadt, Germany) was used according to the suppliers' directions. Stomach mucosa was

exposed and washed with PBS, half of the antral portion of the stomach was immediately placed inside an Eppendorf tube containing the substrate for measurement of urease activity. Absorbance at 550 nm was measured after tubes were incubated for 4 hours at room temperature. The rest of the stomach tissue was stored at -20°C for further treatments. The urease activity values obtained from the stomach of naive mice, which did not undergo immunization or challenge, were used to create a base line to indicate the absence of *H. pylori* infection and therefore protection.

Table 1

15 **UreA and UreB expressing *S. typhimurium* vaccine strains**

Strains	Urease Expression	Source
<i>S. typhimurium</i> SL3261	Negative	Hoiseth and Stocker
<i>S. typhimurium</i> SL3262 pYZ97	Constitutive Low	this study
20 <i>S. typhimurium</i> SL3261::pYZ88pYZ97	High T7-induced expression	this study
<i>S. typhimurium</i> SL3261::pYZ84pYZ97	Medium T7-induced expression	this study
25 <i>S. typhimurium</i> SL3261::pYZ114pYZ97	Low T7-induced expression	this study

Table 2

Mice groups used for immunization

Group	Immunogen	No. of oral immunizations
A	None	0
5 B	PBS oral immunization	1
C	S. typhimurium S3261	1
D	S. typhimurium S3261 pYZ97	1
E	S. typhimurium S3261::pYZ88pYZ97	1
F	S. typhimurium S3261::pYZ84pYZ97	1
10 G	S. typhimurium S3261::pYZ114pYZ97	1
H	Urease B plus cholera toxin	4

15 Results:

In the control mice (groups B and C) 100% infection with H. pylori was observed. In the mice immunized with recombinant attenuated pathogens (groups D, E, F, G) between 0% and 60%
 20 infection (100% to 40% protection) was observed. Immuno-protection did not correlate with humoral anti-UreA and UreB response, suggesting that, in addition to humoral immunity, cellular immunity is critical for protection against H. pylori infection. The results indicate that oral immunization of mice
 25 with UreA and UreB delivered by S. typhimurium attenuated strain is effective to induce high levels of protection against H. pylori colonisation.

In the mice immunized with recombinant urease B plus cholera
 30 toxin considerably higher levels of urease activity were ob-

served under said experimental conditions than when administering the recombinant attenuated pathogens according to the invention.

- s The results of the urease test have been illustrated in table 3.

Table 3

Group	Mouse	$E_{550nm, 4h}$	$E_{4h} - E_{control}$	$E_{con.} \cdot 3$	Dilution
A	1	0,085	-0,022	-0,066	200 μ l+400 μ l
A	2	0,091	-0,016	-0,048	200 μ l+400 μ l
A	3	0,116	0,009	0,027	200 μ l+400 μ l
A	4	0,099	-0,008	-0,024	200 μ l+400 μ l
A	5	0,101	-0,006	-0,018	200 μ l+400 μ l
Control		0,107	0	0	200 μ l+400 μ l
B	1	0,394	0,292	0,876	200 μ l+400 μ l
B	2	0,464	0,362	1,086	200 μ l+400 μ l
B	3	0,329	0,227	0,681	200 μ l+400 μ l
B	4	0,527	0,425	1,275	200 μ l+400 μ l
B	5	0,462	0,36	1,08	200 μ l+400 μ l
Control		0,102	0	0	200 μ l+400 μ l
C	1	0,248	0,145	0,435	200 μ l+400 μ l
C	2	0,369	0,266	0,798	200 μ l+400 μ l
C	3	0,209	0,106	0,318	200 μ l+400 μ l
C	4	0,219	0,116	0,348	200 μ l+400 μ l
C	5	0,24	0,137	0,411	200 μ l+400 μ l
Control		0,103	0	0	200 μ l+400 μ l
D	1	0,143	0,002	0,004	300 μ l+300 μ l
D	2	0,156	0,015	0,03	300 μ l+300 μ l
D	3	0,142	0,001	0,002	300 μ l+300 μ l
D	4	0,114	-0,027	-0,054	300 μ l+300 μ l
D	5	0,133	-0,008	-0,016	300 μ l+300 μ l
Control		0,141	0	0	300 μ l+300 μ l
E	1	0,127	0,027	0,081	200 μ l+400 μ l
E	2	0,094	-0,006	-0,018	200 μ l+400 μ l
E	3	0,099	-0,001	-0,003	200 μ l+400 μ l
E	4	0,161	0,061	0,183	200 μ l+400 μ l
E	5	0,198	0,098	0,294	200 μ l+400 μ l
Control		0,1	0	0	200 μ l+400 μ l
F	1	0,166	0,025	0,05	300 μ l+300 μ l
F	2	0,145	0,004	0,008	300 μ l+300 μ l
F	3	0,166	0,025	0,05	300 μ l+300 μ l
F	4	0,154	0,013	0,026	300 μ l+300 μ l
F	5	0,301	0,16	0,32	300 μ l+300 μ l
Control		0,141	0	0	300 μ l+300 μ l
G	1	0,084	-0,019	-0,057	200 μ l+400 μ l
G	2	0,087	-0,016	-0,048	200 μ l+400 μ l
G	3	0,269	0,166	0,498	200 μ l+400 μ l
G	4	0,085	-0,018	-0,054	200 μ l+400 μ l
G	5	0,092	-0,011	-0,033	200 μ l+400 μ l
Control		0,103	0	0	200 μ l+400 μ l
H	1	0,638	0,531	1,593	200 μ l+400 μ l
H	2	0,282	0,175	0,525	200 μ l+400 μ l
H	3	0,141	0,034	0,102	200 μ l+400 μ l
H	4	0,135	0,028	0,084	200 μ l+400 μ l
H	5	0,171	0,064	0,192	200 μ l+400 μ l
Control		0,107	0	0	200 μ l+400 μ l

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT:
 - (A) NAME: Max-Planck-Gesellschaft zur Foerderung der Wissenschaften e.V. Berlin
 - (B) STREET: Hofgartenstr. 2
 - (C) CITY: Muenchen
 - (E) COUNTRY: Germany
 - (F) POSTAL CODE (ZIP): 80539
- (ii) TITLE OF INVENTION: Helicobacter pylori live vaccine
- (iii) NUMBER OF SEQUENCES: 4
- (iv) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1557 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: both
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Helicobacter pylori
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: alpB
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 1..1554

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

ATG ACA CAA TCT CAA AAA GTA AGA TTC TTA GCC CCT TTA AGC CTA GCG	48
Met Thr Gln Ser Gln Lys Val Arg Phe Leu Ala Pro Leu Ser Leu Ala	
1 5 10 15	
TTA AGC TTG AGC TTC AAT CCA GTG GGC GCT GAA GAA GAT GGG GGC TTT	96
Leu Ser Leu Ser Phe Asn Pro Val Gly Ala Glu Glu Asp Gly Gly Phe	
20 25 30	
ATG ACC TTT GGG TAT GAA TTA GGT CAG GTG GTC CAA CAA GTG AAA AAC	144
Met Thr Phe Gly Tyr Glu Leu Gly Gln Val Val Gln Gln Val Lys Asn	
35 40 45	
CCG GGT AAA ATC AAA GCC GAA GAA TTA GCC GGC TTG TTA AAC TCT ACC	192
Pro Gly Lys Ile Lys Ala Glu Glu Leu Ala Gly Leu Leu Asn Ser Thr	
50 55 60	
ACA ACA AAC AAC ACC AAT ATC AAT ATT GCA GGC ACA GGA GGC AAT GTC	240

Thr 65	Thr	Asn	Asn	Thr	Asn 70	Ile	Asn	Ile	Ala	Gly 75	Thr	Gly	Gly	Asn	Val 80	
GCC Ala	GGG Gly	ACT Thr	TTG Leu	GGC Gly	AAC Asn	CTT Leu	TTT Phe	ATG Met	AAC Asn	CAA Gln	TTA Leu	GGC Gly	AAT Asn	TTG Leu	ATT Ile	288
GAT Asp	TTG Leu	TAT Tyr	CCC Pro	ACT Thr	TTG Leu	AAC Asn	ACT Thr	AGT Ser	AAT Asn	ATC Ile	ACA Thr	CAA Gln	TGT Cys	GGC Gly	ACT Thr	336
ACT Thr	AAT Asn	AGT Ser	GGT Gly	AGT Ser	AGT Ser	AGT Ser	AGT Ser	GGT Gly	GGT Gly	GGT Gly	GCG Ala	GCC Ala	ACA Thr	GCC Ala	GCT Ala	384
GCT Ala	ACT Thr	ACT Thr	AGC Ser	AAT Asn	AAG Lys	CCT Pro	TGT Cys	TTC Phe	CAA Gln	GGT Gly	AAC Asn	CTG Leu	GAT Asp	CTT Leu	TAT Tyr	432
AGA Arg 1	AAA Lys	ATG Met	GTT Val	GAC Asp	TCT Ser	ATC Ile	AAA Lys	ACT Thr	TTG Leu	AGT Ser	CAA Gln	AAC Asn	ATC Ile	AGC Ser	AAG Lys	480
AAT Asn	ATC Ile	TTT Phe	CAA Gln	GGC Gly	AAC Asn	AAC Asn	AAC Asn	ACC Thr	ACG Thr	AGC Ser	CAA Gln	AAT Asn	CTC Leu	TCC Ser	AAC Asn	528
CAG Gln	CTC Leu	AGT Ser	GAG Glu	CTT Leu	AAC Asn	ACC Thr	GCT Ala	AGC Ser	GTT Val	TAT Tyr	TTG Leu	ACT Thr	TAC Tyr	ATG Met	AAC Asn	576
TCG Ser	TTC Phe	TTA Leu	AAC Asn	GCC Ala	AAT Asn	AAC Asn	CAA Gln	GCG Ala	GGT Gly	GGG Gly	ATT Ile	TTT Phe	CAA Gln	AAC Asn	AAC Asn	624
ACT Thr	AAT Asn	CAA Gln	GCT Ala	TAT Tyr	GGA Gly	AAT Asn	GGG Gly	GTT Val	ACC Thr	GCT Ala	CAA Gln	CAA Gln	ATC Ile	GCT Ala	TAT Tyr	672
ATC I 225	CTA Leu	AAG Lys	CAA Gln	GCT Ala	TCA Ser	ATC Ile	ACT Thr	ATG Met	GGG Gly	CCA Pro	AGC Ser	GGT Gly	GAT Asp	AGC Ser	GGT Gly	720
GCT Ala	GCC Ala	GCA Ala	GCG Ala	TTT Phe	TTG Leu	GAT Asp	GCC Ala	GCT Ala	TTA Leu	GCG Ala	CAA Gln	CAT His	GTT Val	TTC Phe	AAC Asn	768
TCC Ser	GCT Ala	AAC Asn	GCC Ala	GGG Gly	AAC Asn	GAT Asp	TTG Leu	AGC Ser	GCT Ala	AAG Lys	GAA Glu	TTC Phe	ACT Thr	AGC Ser	TTG Leu	816
GTG Val	CAA Gln	AAT Asn	ATC Ile	GTC Val	AAT Asn	AAT Asn	TCT Ser	CAA Gln	AAC Asn	GCT Ala	TTA Leu	ACG Thr	CTA Leu	GCC Ala	AAC Asn	864
AAC Asn	GCT Ala	AAC Asn	ATC Ile	AGC Ser	AAT Asn	TCA Ser	ACA Thr	GGC Gly	TAT Tyr	CAA Gln	GTG Val	AGC Ser	TAT Tyr	GGC Gly	GGG Gly	912
AAT Asn	ATT Ile	GAT Asp	CAA Gln	GCG Ala	CGA Arg	TCT Ser	ACC Thr	CAA Gln	CTA Leu	TTA Leu	AAC Asn	AAC Asn	ACC Thr	ACA Thr	AAC Asn	960
ACT	TTG	GCT	AAA	GTT	AGC	GCT	TTG	AAT	AAC	GAG	CTT	AAA	GCT	AAC	CCA	1008

Thr	Leu	Ala	Lys	Val	Ser	Ala	Leu	Asn	Asn	Glu	Leu	Lys	Ala	Asn	Pro		
				325					330					335			
TGG	CTT	GGG	AAT	TTT	GCC	GCC	GGT	AAC	AGC	TCT	CAA	GTG	AAT	GCG	TTT		1056
Trp	Leu	Gly	Asn	Phe	Ala	Ala	Gly	Asn	Ser	Ser	Gln	Val	Asn	Ala	Phe		
			340					345					350				
AAC	GGG	TTT	ATC	ACT	AAA	ATC	GGT	TAC	AAG	CAA	TTC	TTT	GGG	GAA	AAC		1104
Asn	Gly	Phe	Ile	Thr	Lys	Ile	Gly	Tyr	Lys	Gln	Phe	Phe	Gly	Glu	Asn		
			355				360					365					
AAG	AAT	GTG	GGC	TTA	CGC	TAC	TAC	GGC	TTC	TTC	AGC	TAT	AAC	GGC	GCG		1152
Lys	Asn	Val	Gly	Leu	Arg	Tyr	Tyr	Gly	Phe	Phe	Ser	Tyr	Asn	Gly	Ala		
			370			375					380						
GGC	GTG	GGT	AAT	GGC	CCT	ACT	TAC	AAT	CAA	GTC	AAT	TTG	CTC	ACT	TAT		1200
Gly	Val	Gly	Asn	Gly	Pro	Thr	Tyr	Asn	Gln	Val	Asn	Leu	Leu	Thr	Tyr		
385					390					395					400		
GGG	GTG	GGG	ACT	GAT	GTG	CTT	TAC	AAT	GTG	TTT	AGC	CGC	TCT	TTT	GGT		1248
Gly	Val	Gly	Thr	Asp	Val	Leu	Tyr	Asn	Val	Phe	Ser	Arg	Ser	Phe	Gly		
				405					410					415			
TAGT	AGG	AGT	CTT	AAT	GCG	GGC	TTC	TTT	GGG	GGG	ATC	CAA	CTC	GCA	GGG		1296
Ser	Arg	Ser	Leu	Asn	Ala	Gly	Phe	Phe	Gly	Gly	Ile	Gln	Leu	Ala	Gly		
			420					425					430				
GAT	ACT	TAC	ATC	AGC	ACG	CTA	AGA	AAC	AGC	TCT	CAG	CTT	GCG	AGC	AGA		1344
Asp	Thr	Tyr	Ile	Ser	Thr	Leu	Arg	Asn	Ser	Ser	Gln	Leu	Ala	Ser	Arg		
			435				440					445					
CCT	ACA	GCG	ACG	AAA	TTC	CAA	TTC	TTG	TTT	GAT	GTG	GGC	TTA	CGC	ATG		1392
Pro	Thr	Ala	Thr	Lys	Phe	Gln	Phe	Leu	Phe	Asp	Val	Gly	Leu	Arg	Met		
			450			455					460						
AAC	TTT	GGT	ATC	TTG	AAA	AAA	GAC	TTG	AAA	AGC	CAT	AAC	CAG	CAT	TCT		1440
Asn	Phe	Gly	Ile	Leu	Lys	Lys	Asp	Leu	Lys	Ser	His	Asn	Gln	His	Ser		
					470					475					480		
ATA	GAA	ATC	GGT	GTG	CAA	ATC	CCT	ACG	ATT	TAC	AAC	ACT	TAC	TAT	AAA		1488
	Glu	Ile	Gly	Val	Gln	Ile	Pro	Thr	Ile	Tyr	Asn	Thr	Tyr	Tyr	Lys		
				485				490						495			
GCT	GGC	GGT	GCT	GAA	GTG	AAA	TAC	TTC	CGC	CCT	TAT	AGC	GTG	TAT	TGG		1536
Ala	Gly	Gly	Ala	Glu	Val	Lys	Tyr	Phe	Arg	Pro	Tyr	Ser	Val	Tyr	Trp		
			500					505					510				
GTC	TAT	GGC	TAC	GCC	TTC	TAA											1557
Val	Tyr	Gly	Tyr	Ala	Phe												
			515														

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 518 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met	Thr	Gln	Ser	Gln	Lys	Val	Arg	Phe	Leu	Ala	Pro	Leu	Ser	Leu	Ala		
1				5					10					15			

Leu	Ser	Leu	Ser	Phe	Asn	Pro	Val	Gly	Ala	Glu	Glu	Asp	Gly	Gly	Phe	
			20					25					30			
Met	Thr	Phe	Gly	Tyr	Glu	Leu	Gly	Gln	Val	Val	Gln	Gln	Val	Lys	Asn	
		35					40					45				
Pro	Gly	Lys	Ile	Lys	Ala	Glu	Glu	Leu	Ala	Gly	Leu	Leu	Asn	Ser	Thr	
	50					55					60					
Thr	Thr	Asn	Asn	Thr	Asn	Ile	Asn	Ile	Ala	Gly	Thr	Gly	Gly	Asn	Val	
	65				70					75					80	
Ala	Gly	Thr	Leu	Gly	Asn	Leu	Phe	Met	Asn	Gln	Leu	Gly	Asn	Leu	Ile	
				85					90					95		
Asp	Leu	Tyr	Pro	Thr	Leu	Asn	Thr	Ser	Asn	Ile	Thr	Gln	Cys	Gly	Thr	
			100					105					110			
Thr	Asn	Ser	Gly	Ser	Ser	Ser	Ser	Gly	Gly	Gly	Ala	Ala	Thr	Ala	Ala	
		115					120					125				
Ala	Thr	Thr	Ser	Asn	Lys	Pro	Cys	Phe	Gln	Gly	Asn	Leu	Asp	Leu	Tyr	
	130					135					140					
Arg	Lys	Met	Val	Asp	Ser	Ile	Lys	Thr	Leu	Ser	Gln	Asn	Ile	Ser	Lys	
	145				150					155					160	
Asn	Ile	Phe	Gln	Gly	Asn	Asn	Asn	Thr	Thr	Ser	Gln	Asn	Leu	Ser	Asn	
			165					170						175		
Gln	Leu	Ser	Glu	Leu	Asn	Thr	Ala	Ser	Val	Tyr	Leu	Thr	Tyr	Met	Asn	
			180					185					190			
Ser	Phe	Leu	Asn	Ala	Asn	Asn	Gln	Ala	Gly	Gly	Ile	Phe	Gln	Asn	Asn	
		195					200					205				
Thr	Asn	Gln	Ala	Tyr	Gly	Asn	Gly	Val	Thr	Ala	Gln	Gln	Ile	Ala	Tyr	
	210					215					220					
e	Leu	Lys	Gln	Ala	Ser	Ile	Thr	Met	Gly	Pro	Ser	Gly	Asp	Ser	Gly	
	225				230					235					240	
Ala	Ala	Ala	Ala	Phe	Leu	Asp	Ala	Ala	Leu	Ala	Gln	His	Val	Phe	Asn	
				245					250					255		
Ser	Ala	Asn	Ala	Gly	Asn	Asp	Leu	Ser	Ala	Lys	Glu	Phe	Thr	Ser	Leu	
			260					265					270			
Val	Gln	Asn	Ile	Val	Asn	Asn	Ser	Gln	Asn	Ala	Leu	Thr	Leu	Ala	Asn	
		275					280					285				
Asn	Ala	Asn	Ile	Ser	Asn	Ser	Thr	Gly	Tyr	Gln	Val	Ser	Tyr	Gly	Gly	
	290					295					300					
Asn	Ile	Asp	Gln	Ala	Arg	Ser	Thr	Gln	Leu	Leu	Asn	Asn	Thr	Thr	Asn	
	305				310					315					320	
Thr	Leu	Ala	Lys	Val	Ser	Ala	Leu	Asn	Asn	Glu	Leu	Lys	Ala	Asn	Pro	
			325						330					335		
Trp	Leu	Gly	Asn	Phe	Ala	Ala	Gly	Asn	Ser	Ser	Gln	Val	Asn	Ala	Phe	
			340					345					350			

Asn Gly Phe Ile Thr Lys Ile Gly Tyr Lys Gln Phe Phe Gly Glu Asn
 355 360 365
 Lys Asn Val Gly Leu Arg Tyr Tyr Gly Phe Phe Ser Tyr Asn Gly Ala
 370 375 380
 Gly Val Gly Asn Gly Pro Thr Tyr Asn Gln Val Asn Leu Leu Thr Tyr
 385 390 395 400
 Gly Val Gly Thr Asp Val Leu Tyr Asn Val Phe Ser Arg Ser Phe Gly
 405 410 415
 Ser Arg Ser Leu Asn Ala Gly Phe Phe Gly Gly Ile Gln Leu Ala Gly
 420 425 430
 Asp Thr Tyr Ile Ser Thr Leu Arg Asn Ser Ser Gln Leu Ala Ser Arg
 435 440 445
 Pro Thr Ala Thr Lys Phe Gln Phe Leu Phe Asp Val Gly Leu Arg Met
 450 455 460
 A Phe Gly Ile Leu Lys Lys Asp Leu Lys Ser His Asn Gln His Ser
 465 470 475 480
 Ile Glu Ile Gly Val Gln Ile Pro Thr Ile Tyr Asn Thr Tyr Tyr Lys
 485 490 495
 Ala Gly Gly Ala Glu Val Lys Tyr Phe Arg Pro Tyr Ser Val Tyr Trp
 500 505 510
 Val Tyr Gly Tyr Ala Phe
 515

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1557 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Helicobacter pylori*

(vii) IMMEDIATE SOURCE:

- (B) CLONE: alpA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1554

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

ATG ATA AAA AAG AAT AGA ACG CTG TTT CTT AGT CTA GCC CTT TGC GCT
 Met Ile Lys Lys Asn Arg Thr Leu Phe Leu Ser Leu Ala Leu Cys Ala
 520 525 530
 AGC ATA AGT TAT GCC GAA GAT GAT GGA GGG TTT TTC ACC GTC GGT TAT
 Ser Ile Ser Tyr Ala Glu Asp Asp Gly Gly Phe Phe Thr Val Gly Tyr
 535 540 545 550

48

96

CAG	CTC	GGG	CAA	GTC	ATG	CAA	GAT	GTC	CAA	AAC	CCA	GGG	GGG	GCT	AAA		44
Gln	Leu	Gly	Gln	Val	Met	Gln	Asp	Val	Gln	Asn	Pro	Gly	Gly	Ala	Lys		
				555					560					565			
AGC	GAC	GAA	CTC	GCC	AGA	GAG	CTT	AAC	GCT	GAT	GTA	ACG	AAC	AAC	ATT		192
Ser	Asp	Glu	Leu	Ala	Arg	Glu	Leu	Asn	Ala	Asp	Val	Thr	Asn	Asn	Ile		
			570					575					580				
TTA	AAC	AAC	AAC	ACC	GGA	GGC	AAC	ATC	GCA	GGG	GCG	TTG	AGT	AAC	GCT		240
Leu	Asn	Asn	Asn	Thr	Gly	Gly	Asn	Ile	Ala	Gly	Ala	Leu	Ser	Asn	Ala		
			585				590					595					
TTC	TCC	CAA	TAC	CTT	TAT	TCG	CTT	TTA	GGG	GCT	TAC	CCC	ACA	AAA	CTC		288
Phe	Ser	Gln	Tyr	Leu	Tyr	Ser	Leu	Leu	Gly	Ala	Tyr	Pro	Thr	Lys	Leu		
	600					605					610						
AAT	GGT	AGC	GAT	GTG	TCT	GCG	AAC	GCT	CTT	TTA	AGT	GGT	GCG	GTA	GGC		336
Asn	Gly	Ser	Asp	Val	Ser	Ala	Asn	Ala	Leu	Leu	Ser	Gly	Ala	Val	Gly		
	615				620					625					630		
TCT	GGG	ACT	TGT	GCG	GCT	GCA	GGG	ACG	GCT	GGT	GGC	ACT	TCT	CTT	AAC		384
S	Gly	Thr	Cys	Ala	Ala	Ala	Gly	Thr	Ala	Gly	Gly	Thr	Ser	Leu	Asn		
				635					640					645			
ACT	CAA	AGC	ACT	TGC	ACC	GTT	GCG	GGC	TAT	TAC	TGG	CTC	CCT	AGC	TTG		432
Thr	Gln	Ser	Thr	Cys	Thr	Val	Ala	Gly	Tyr	Tyr	Trp	Leu	Pro	Ser	Leu		
			650					655					660				
ACT	GAC	AGG	ATT	TTA	AGC	ACG	ATC	GGC	AGC	CAG	ACT	AAC	TAC	GGC	ACG		480
Thr	Asp	Arg	Ile	Leu	Ser	Thr	Ile	Gly	Ser	Gln	Thr	Asn	Tyr	Gly	Thr		
		665					670					675					
AAC	ACC	AAT	TTC	CCC	AAC	ATG	CAA	CAA	CAG	CTC	ACC	TAC	TTG	AAT	GCG		528
Asn	Thr	Asn	Phe	Pro	Asn	Met	Gln	Gln	Gln	Leu	Thr	Tyr	Leu	Asn	Ala		
			680			685					690						
GGG	AAT	GTG	TTT	TTT	AAT	GCG	ATG	AAT	AAG	GCT	TTA	GAG	AAT	AAG	AAT		576
Gly	Asn	Val	Phe	Phe	Asn	Ala	Met	Asn	Lys	Ala	Leu	Glu	Asn	Lys	Asn		
	695				700					705					710		
C	ACT	AGT	AGT	GCT	AGT	GGA	ACT	AGT	GGT	GCG	ACT	GGT	TCA	GAT	GGT		624
Gly	Thr	Ser	Ser	Ala	Ser	Gly	Thr	Ser	Gly	Ala	Thr	Gly	Ser	Asp	Gly		
				715					720					725			
CAA	ACT	TAC	TCC	ACA	CAA	GCT	ATC	CAA	TAC	CTT	CAA	GGC	CAA	CAA	AAT		672
Gln	Thr	Tyr	Ser	Thr	Gln	Ala	Ile	Gln	Tyr	Leu	Gln	Gly	Gln	Gln	Asn		
			730					735					740				
ATC	TTA	AAT	AAC	GCA	GCG	AAC	TTG	CTC	AAG	CAA	GAT	GAA	TTG	CTC	TTA		720
Ile	Leu	Asn	Asn	Ala	Ala	Asn	Leu	Leu	Lys	Gln	Asp	Glu	Leu	Leu	Leu		
			745				750					755					
GAA	GCT	TTC	AAC	TCT	GCC	GTA	GCC	GCC	AAC	ATT	GGG	AAT	AAG	GAA	TTC		768
Glu	Ala	Phe	Asn	Ser	Ala	Val	Ala	Ala	Asn	Ile	Gly	Asn	Lys	Glu	Phe		
		760				765					770						
AAT	TCA	GCC	GCT	TTT	ACA	GGT	TTG	GTG	CAA	GGC	ATT	ATT	GAT	CAA	TCT		816
Asn	Ser	Ala	Ala	Phe	Thr	Gly	Leu	Val	Gln	Gly	Ile	Ile	Asp	Gln	Ser		
					780					785					790		
CAA	GCG	GTT	TAT	AAC	GAG	CTC	ACT	AAA	AAC	ACC	ATT	AGC	GGG	AGT	GCG		864
Gln	Ala	Val	Tyr	Asn	Glu	Leu	Thr	Lys	Asn	Thr	Ile	Ser	Gly	Ser	Ala		
				795					800					805			

GTT ATT AGC GCT GGG ATA AAC TCC AAC CAA GCT AAC GCT GTG CAA GGG Val Ile Ser Ala Gly Ile Asn Ser Asn Gln Ala Asn Ala Val Gln Gly 810 815 820	912
CGC GCT AGT CAG CTC CCT AAC GCT CTT TAT AAC GCG CAA GTA ACT TTG Arg Ala Ser Gln Leu Pro Asn Ala Leu Tyr Asn Ala Gln Val Thr Leu 825 830 835	960
GAT AAA ATC AAT GCG CTC AAT AAT CAA GTG AGA AGC ATG CCT TAC TTG Asp Lys Ile Asn Ala Leu Asn Asn Gln Val Arg Ser Met Pro Tyr Leu 840 845 850	1008
CCC CAA TTC AGA GCC GGG AAC AGC CGT TCA ACG AAT ATT TTA AAC GGG Pro Gln Phe Arg Ala Gly Asn Ser Arg Ser Thr Asn Ile Leu Asn Gly 855 860 865 870	1056
TTT TAC ACC AAA ATA GGC TAT AAG CAA TTC TTC GGG AAG AAA AGG AAT Phe Tyr Thr Lys Ile Gly Tyr Lys Gln Phe Phe Gly Lys Lys Arg Asn 875 880 885	1104
ATC GGT TTG CGC TAT TAT GGT TTC TTT TCT TAT AAC GGA GCG AGC GTG Gly Leu Arg Tyr Tyr Gly Phe Phe Ser Tyr Asn Gly Ala Ser Val 890 895 900	1152
GGC TTT AGA TCC ACT CAA AAT AAT GTA GGG TTA TAC ACT TAT GGG GTG Gly Phe Arg Ser Thr Gln Asn Asn Val Gly Leu Tyr Thr Tyr Gly Val 905 910 915	1200
GGG ACT GAT GTG TTG TAT AAC ATC TTT AGC CGC TCC TAT CAA AAC CGC Gly Thr Asp Val Leu Tyr Asn Ile Phe Ser Arg Ser Tyr Gln Asn Arg 920 925 930	1248
TCT GTG GAT ATG GGC TTT TTT AGC GGT ATC CAA TTA GCC GGT GAG ACC Ser Val Asp Met Gly Phe Phe Ser Gly Ile Gln Leu Ala Gly Glu Thr 935 940 945 950	1296
TTC CAA TCC ACG CTC AGA GAT GAC CCC AAT GTG AAA TTG CAT GGG AAA Phe Gln Ser Thr Leu Arg Asp Asp Pro Asn Val Lys Leu His Gly Lys 955 960 965	1344
AAT AAC ACG CAC TTC CAG TTC CTC TTT GAC TTC GGT ATG AGG ATG Ile Asn Asn Thr His Phe Gln Phe Leu Phe Asp Phe Gly Met Arg Met 970 975 980	1392
AAC TTC GGT AAG TTG GAC GGG AAA TCC AAC CGC CAC AAC CAG CAC ACG Asn Phe Gly Lys Leu Asp Gly Lys Ser Asn Arg His Asn Gln His Thr 985 990 995	1440
GTG GAA TTT GGC GTA GTG GTG CCT ACG ATT TAT AAC ACT TAT TAC AAA Val Glu Phe Gly Val Val Val Pro Thr Ile Tyr Asn Thr Tyr Tyr Lys 1000 1005 1010	1488
TCA GCA GGG ACT ACC GTG AAG TAT TTC CGT CCT TAT AGC GTT TAT TGG Ser Ala Gly Thr Thr Val Lys Tyr Phe Arg Pro Tyr Ser Val Tyr Trp 1015 1020 1025 1030	1536
TCT TAT GGG TAT TCA TTC TAA Ser Tyr Gly Tyr Ser Phe 1035	1557

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 518 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Ile Lys Lys Asn Arg Thr Leu Phe Leu Ser Leu Ala Leu Cys Ala
1 5 10 15
Ser Ile Ser Tyr Ala Glu Asp Asp Gly Gly Phe Phe Thr Val Gly Tyr
20 25 30
Gln Leu Gly Gln Val Met Gln Asp Val Gln Asn Pro Gly Gly Ala Lys
35 40 45
Ser Asp Glu Leu Ala Arg Glu Leu Asn Ala Asp Val Thr Asn Asn Ile
50 55 60
Leu Asn Asn Asn Thr Gly Gly Asn Ile Ala Gly Ala Leu Ser Asn Ala
65 70 75 80
Phe Ser Gln Tyr Leu Tyr Ser Leu Leu Gly Ala Tyr Pro Thr Lys Leu
85 90 95
Asn Gly Ser Asp Val Ser Ala Asn Ala Leu Leu Ser Gly Ala Val Gly
100 105 110
Ser Gly Thr Cys Ala Ala Ala Gly Thr Ala Gly Gly Thr Ser Leu Asn
115 120 125
Thr Gln Ser Thr Cys Thr Val Ala Gly Tyr Tyr Trp Leu Pro Ser Leu
130 135 140
Thr Asp Arg Ile Leu Ser Thr Ile Gly Ser Gln Thr Asn Tyr Gly Thr
145 150 155 160
Asn Thr Asn Phe Pro Asn Met Gln Gln Gln Leu Thr Tyr Leu Asn Ala
165 170 175
c Asn Val Phe Phe Asn Ala Met Asn Lys Ala Leu Glu Asn Lys Asn
180 185 190
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Glu Ala Phe Asn Ser Ala Val Ala Ala Asn Ile Gly Asn Lys Glu Phe
245 250 255
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Gln Ala Val Tyr Asn Glu Leu Thr Lys Asn Thr Ile Ser Gly Ser Ala
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Arg Ala Ser Gln Leu Pro Asn Ala Leu Tyr Asn Ala Gln Val Thr Leu
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Asp Lys Ile Asn Ala Leu Asn Asn Gln Val Arg Ser Met Pro Tyr Leu
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Pro Gln Phe Arg Ala Gly Asn Ser Arg Ser Thr Asn Ile Leu Asn Gly
 340 345 350

Phe Tyr Thr Lys Ile Gly Tyr Lys Gln Phe Phe Gly Lys Lys Arg Asn
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Ile Gly Leu Arg Tyr Tyr Gly Phe Phe Ser Tyr Asn Gly Ala Ser Val
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Gly Phe Arg Ser Thr Gln Asn Asn Val Gly Leu Tyr Thr Tyr Gly Val
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Gly Thr Asp Val Leu Tyr Asn Ile Phe Ser Arg Ser Tyr Gln Asn Arg
 405 410 415

S Val Asp Met Gly Phe Phe Ser Gly Ile Gln Leu Ala Gly Glu Thr
 420 425 430

Phe Gln Ser Thr Leu Arg Asp Asp Pro Asn Val Lys Leu His Gly Lys
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Ile Asn Asn Thr His Phe Gln Phe Leu Phe Asp Phe Gly Met Arg Met
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Asn Phe Gly Lys Leu Asp Gly Lys Ser Asn Arg His Asn Gln His Thr
 465 470 475 480

Val Glu Phe Gly Val Val Val Pro Thr Ile Tyr Asn Thr Tyr Tyr Lys
 485 490 495

Ser Ala Gly Thr Thr Val Lys Tyr Phe Arg Pro Tyr Ser Val Tyr Trp
 500 505 510

Ser Tyr Gly Tyr Ser Phe
 515

Patent Claims

1. A recombinant attenuated microbial pathogen, which comprises at least one heterologous nucleic acid molecule encoding a Helicobacter antigen, wherein said pathogen is capable to express said nucleic acid molecule or capable to cause the expression of said nucleic acid molecule in a target cell.
2. The pathogen according to claim 1, which is an enterobacterial cell, especially a Salmonella cell.
3. The pathogen according to claim 1 or 2, which is a Salmonella aro mutant cell.
4. The pathogen according to any of claims 1-3, wherein the Helicobacter antigen is urease, a urease subunit, an immunologically reactive fragment thereof, or a peptide mimotope thereof.
5. The pathogen according to any one of claims 1-3, wherein the Helicobacter antigen is a secretory polypeptide from Helicobacter, an immunologically reactive fragment thereof, or a peptide mimotope thereof.
6. The pathogen according to any one of claims 1-3 and 5, wherein the Helicobacter antigen is selected from the group consisting of the antigens AlpA, AlpB, immunologically reactive fragments thereof, or a peptide mimotope thereof.
7. The pathogen according to any one of claims 1-6, wherein said nucleic acid molecule encoding a Helicobacter antigen is capable to be expressed phase variably.
8. The pathogen according to claim 7,

wherein said nucleic acid molecule encoding the Helicobacter antigen is under control of an expression signal which is substantially inactive in the pathogen and which is capable to be activated by a nucleic acid reorganization caused by a nucleic acid reorganization mechanism in the pathogen.

9. The pathogen according to claim 8, wherein the expression signal is a bacteriophage promoter, and the activation is caused by a DNA reorganization resulting in the production of a corresponding bacteriophage RNA polymerase in the pathogen.
10. The pathogen according to any one of claims 1-9, further comprising at least one second nucleic acid molecule encoding an immunomodulatory polypeptide, wherein said pathogen is capable to express said second nucleic acid molecule.
11. Pharmaceutical composition comprising as an active agent a recombinant attenuated pathogen according to any one of claims 1-10, optionally together with pharmaceutically acceptable diluents, carriers and adjuvants.
12. Composition according to claim 11, which is a living vaccine, which is suitable for administration to a mucosal surface or via the parenteral route.
13. A method for the preparation of a living vaccine comprising formulating an attenuated pathogen according to any one of claims 1-10 in a pharmaceutically effective amount with pharmaceutically acceptable diluents, carriers and/or adjuvants.
14. A method for preparing a recombinant attenuated pathogen according to any one of claims 1-10, comprising the steps:

- a) inserting a nucleic acid molecule encoding a Helicobacter antigen into an attenuated pathogen, wherein a recombinant attenuated pathogen is obtained, which is capable of expressing said nucleic acid molecule or is capable to cause expression of said nucleic acid molecule in a target cell, and
- b) cultivating said recombinant attenuated pathogen under suitable conditions.

15. The method according to claim 15, wherein said nucleic acid molecule encoding a Helicobacter antigen is located on an extrachromosomal plasmid.

16. A method for identifying Helicobacter antigens, which raise a protective immune response in a mammalian host, comprising the steps of:

- a) providing an expression gene bank of Helicobacter in an attenuated pathogen and
- b) screening the clones of the gene bank for their ability to confer protective immunity against a Helicobacter infection in a mammalian host.

Abstract

The present invention relates to novel recombinant live vaccines, which provide protective immunity against an infection by *Helicobacter pylori* and a method of screening *H. pylori* antigens for optimized vaccines.

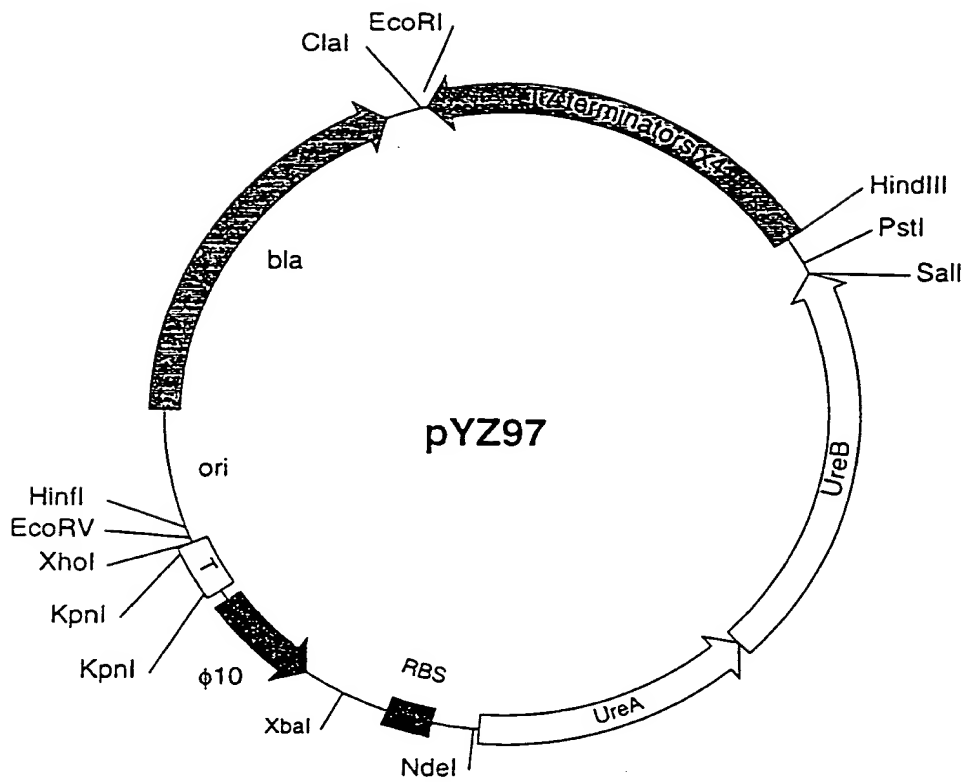
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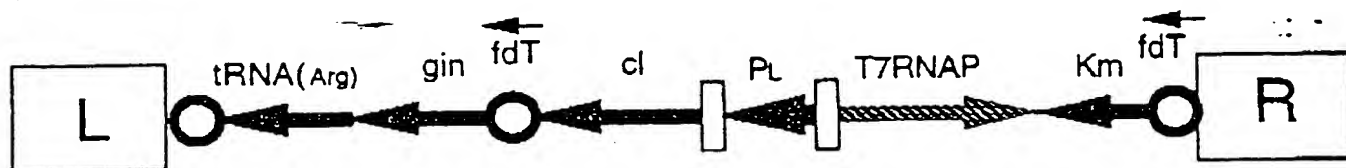
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FIG. 1

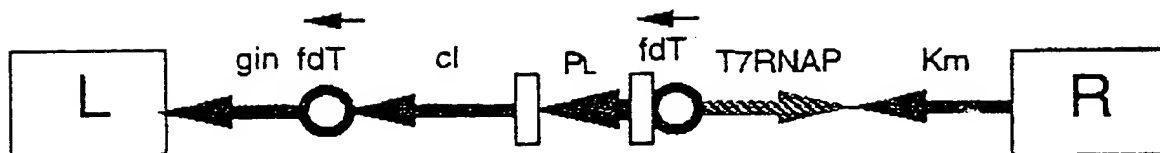


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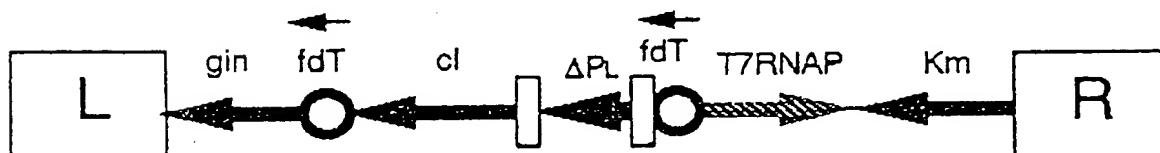
FIG. 2



pYZ88 (high expression)



pYZ84 (medium expression)



pYZ114 (low expression)